NOTES ON A FRESH-WATER MEDUSA FOUND IN STALLWORTH LAKE, TUSCALOOSA, ALABAMA

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During a series of collecting trips, fresh-water medusæ were found in Stallworth Lake near Tuscaloosa, Alabama (White, 1929). They were first observed on September 14, 1928. I visited the lake at least twice a week and never failed to find medusæ until the ninth of October. They did not reappear after this date, although I continued to visit the lake at regular intervals. The approach of cold weather does not seem to have caused their disappearance, as has been suggested in other cases, since warm weather persisted for several weeks after they were last seen. Specimens, which I had placed in large jars of pond water in the laboratory, lived for about two weeks longer, gradually wasting away in size and finally dying. The water contained small Crustacea, so it is hardly possible that lack of food caused their death.

The medusæ were sensitive to changes in intensity of light and to disturbances in the water. In general they were more abundant at the surface on bright days than on cloudy ones. Although I could not bring the medusæ to the surface of the lake at night by shining a flashlight on the water, they became very active when the lights were suddenly turned on in a dark laboratory. I have brought them to the surface by violently thrashing the water at a considerable depth with a long board or iron pipe. They also became very active when the water of the aquarium was stirred.

No hydroid stages were found although repeated searches were made for them from time to time during the fall of 1928 and the spring of 1929. Quantities of scrapings from the piles of the pier and the surfaces of submerged boards and rocks were examined. The hydroid stage was probably present, however, and I believe that if a more extensive search had been made, polyps would have been found in considerable numbers, since the medusæ were so abundant. Perhaps the hydroids were confined to a few localized areas, which would account for my not finding them.

The medusæ have not been observed in the lake since October, 1928. I examined the lake at frequent intervals during the entire summer of 1929 but did not see a single medusa.

The Lake

Stallworth Lake is situated on a terrace midway between the business district of Tuscaloosa, Alabama, and the Warrior River. It was formed in 1918 by damming up four acres of marshy land and is supplied by springs which keep a small stream running from the lake. This streamlet flows into a small pond which finally empties into the river. The depth of the lake varies from a few inches to about twenty feet. Along the edges the bottom is sandy, but in the deepest parts it is covered with mud. There is an abundance of plant life in the lake. Fresh-water algæ give the water a greenish color. Several species of the higher plants are also present. Willows line the dam on one side of the lake. The fauna of the lake is abundant and varied. Numerous species of Protozoa and members of the other invertebrate phyla were constantly encountered during the search which was undertaken for the hydroid. Fish have been introduced from hatcheries in Mississippi and the Warrior River. A large number of turtles are found in the lake, no doubt coming in from the river. Mussels of the genus *Lampsilis* abound in the lake as do snails of the genus *Physa*. In the spring and early summer the lake is used as a swimming pool. Later it is little frequented due to the rather slow change of water. There is a pier, extending out over the water from one shore, which connects the boat house with a small island. A part of this pier is covered by a roof. Most of the specimens were collected from the water on the sides of this pier.

Although the Warrior River is subject to sudden rises which spread it over the surrounding territory, the level of the river has never reached that of the lake. The lake has never been drained, but every year the water is lowered about two feet to facilitate cleaning and repairing. The pH of the water at the time the medusæ were found was 7.2. As for the temperature of the lake, it never freezes, and in the summer the surface may reach a temperature of eighty-five degrees Fahrenheit, due to the very slow turnover of the water.

Historical Survey of the Fresh-water Medusæ

Although marine medusæ have been known for centuries (Aristotle mentions several species), a fresh-water medusa was not reported until 1880. In June of that year Mr. Sowerby, secretary of the Royal Botanical Society, found medusæ in the Society's *Victoria regia* tank in Regent's Park, London. He gave specimens to Lankester and Allman who studied and described them. Allman proposed the name *Limnocodium victoria* for the new form, while Lankester called it *Craspedacusta sowerbii*, in allusion to the relation of the otocysts to the velum. For

a number of years the medusa was known as *Limnocodium sowerbii*, which evidently violates the accepted rules of nomenclature. An attempt was made to have this name validated, but the petition was denied by the International Commission on Zoölogical Nomenclature (Mayer, 1910). Lankester's name, *Craspedacusta sowerbii*, published on June 17, 1880, has priority, and is clearly the correct one. I call attention to this point since the name *Limnocodium* has appeared in the literature within the last two years. *Craspedacusta sowerbii* appeared in the lily-tank of Regent's Park for a number of years, finally disappearing in 1893. Romanes (1880) reported some interesting experiments on the physiology of this fresh-water medusa. Bourne (1884) first described the hydroid stage. Fowler (1890) described medusoid bud formation and gave a complete bibliography of the literature up to that time. Günther (1894) worked out the histology of the medusa stage of this form.

In the last half-century fresh-water medusæ have been reported many times from widely separated localities. Edward Potts (1897) reported the first fresh-water species from America. He had already found the hydroid stage of this form (1885) and had called it *Microhydra ryderi*. Ryder (1885), believing that *Microhydra ryderi* was probably the hydroid stage of a medusa and that this medusa would prove to be generically different from *Craspedacusta*, because of certain differences between *Microhydra ryderi* and the hydroid stage of *C. sowerbii*, proposed the generic name Pottsia, should the medusa stage be found. Why he should have favored separate names for the hydroid and the medusa is not clear. At any rate the name was not valid and was never used. Payne (1924) has demonstrated that *Microhydra ryderi* is a species of the genus *Craspedacusta*, and has designated it *Craspedacusta ryderi*, abolishing the genus *Microhydra*. He reported the complete life cycle of this species (1926).

Although the evidence is not conclusive and only a study of the development could permit a final decision, presumably, the medusæ found by Hargitt (1907), Coker (Payne, 1924), and Garman (1916), and assigned to the European species *Craspedacusta sowerbii*, are specifically identical with the forms found in Boss Lake, Indiana (Payne, 1924) and in Stallworth Lake, Alabama, and should be referred to the American species, *Craspedacusta ryderi*.

Roch (1924) found fresh-water medusæ in a mill stream near Berlin. The largest specimens measured 0.68 millimeter in diameter. They differed from the young medusa of *Craspedacusta ryderi* (then called *Microhydra ryderi*) in having sixteen tentacles which were not of uniform length and which did not appear simultaneously in the course

of the development of the medusa. He also stated that the great geographical separation of this form from *Microhydra ryderi* spoke little for their identity. To this form he gave the name *Microhydra germanica*. It is, no doubt, a member of the genus *Craspedacusta*, and whether it is a different species from *Craspedacusta sowerbii*, the European form, can only be determined by a study of the hydroid, budding, and medusoid bud formation. If, indeed, it is a different species, the correct name would be *Craspedacusta germanica*.

Other species of fresh-water medusæ which have been reported up to the present time are *Limnocnida tanganjicæ* (Günther, 1893) from Africa, *Craspedacusta kawaii* (Oka, 1908) from China, and *Limnocnida indica* (Annandale, 1912) from India. Payne (1924, 1926) gives an extensive bibliography covering fresh-water medusæ to that date. Other papers not listed are cited here. These include a report by Goette (1908), who found them in Strassburg. Pelosse (1919) found them in a park in Lyon. Backhoff (1924) reported them from Stettin. Flower and Lockyer (1928) reported the reappearance of *Craspedacusta sowerbii* in the Royal Botanic Society's garden in Regent's Park, London, where fresh-water medusæ were first reported in 1880. Rupert Vallentin (1930) found medusæ in the Exeter ship canal in July of 1928 and 1929.

The Adult Medusa

Payne (1924) has given an excellent description of the medusa of *Craspedacusta ryderi*. Since the medusæ of Stallworth Lake conform very closely to his description, I shall give here merely a few details wherein they differ from those found in Boss Lake. Payne found that the sexes were so alike that the only way to tell them apart was by an examination of the gonads. I found only males, although a number of the medusæ were examined by smear and sectioning methods. I am inclined to think that if females were present, at all, they occurred in very small numbers. As I did not arrive in Tuscaloosa until early fall, all the medusæ were fairly large—none measuring less than fourteen millimeters in diameter. The medusæ are, evidently, slightly larger than those found by Payne, the largest specimens measuring about twenty millimeters in diameter, when in the relaxed condition. There is a correspondingly greater number of tentacles—over four hundred were counted on each of several individuals. Although the tentacles of *Craspedacusta* have, heretofore, been placed in three size groups, they seem to fall into four fairly distinct sets. Allman (1880) shows this clearly in his drawing, but uses only three groups in his description. First, there are the perradials, four large tentacles lying

at the terminations of the radial canals. Those of the next group are almost as large and lie between the perradials. There are about twenty-five of these. The third set is considerably smaller than the first two. There are about thirty-five in this group, which are scattered among the larger tentacles. The smallest tentacles form a hair-like fringe around the periphery of the umbrella. They are so numerous that they are difficult to count. Three hundred and thirty-eight were counted on one individual. Payne (1924) found sixty-nine lithocysts in a specimen five millimeters in diameter. I have counted over two hundred in several mature specimens.

These differences may have been caused by developmental differences or by dissimiliar environmental conditions. In other respects the medusæ from the two localities are quite similar. The study of the hydroid, if it is found, and the developmental processes may, possibly, bring out specific differences between the Alabama and Indiana forms, but at the present time I am inclined to think that the medusæ are specifically identical and have tentatively assigned the form from Stallworth Lake to the species *Craspedacusta ryderi*.

Spermatogenesis

When the medusæ were first observed specimens were taken to the laboratory. The gonads of a number of these were excised and fixed in Bouin's fluid. Sections of these gonads were cut at five microns and stained with iron hæmatoxylin. Others were mordanted in Flemming's fixative and stained with Flemming's triple stain. These sections demonstrated that all of the medusæ examined were males. In spite of the fact that all of the medusæ were fully grown, various stages of spermatogenesis were observed in each section. Since no detailed study of spermatogenesis in a fresh-water medusa has been reported, certain observations will be given here.

From the subumbrella side of each radial canal there projects a long sac-like pouch, lined by a layer of entodermal epithelium, a single cell in thickness, which is continuous with that of the radial canal and which forms the cavity of the gonad. The mesogloea is much reduced in the gonad, forming only a very thin layer at the base of the entoderm cells. The rest of the wall of the gonad is thick and composed of germ cells and developing sperm, with a thin superficial covering of ectodermal tissue. The entoderm cells are columnar in shape with large irregular vacuoles in their cytoplasm. The nuclei are found in the end of the cells distal to the cavity of the gonad. They contain large centrally placed nucleoli. Adjacent to this entodermal layer are the spermatogonia. Since only mature gonads were secured, no in-

formation can be given concerning the origin of the germ cells. Günther (1894) considered them to be of ectodermal origin. He distinguished sperm mother cells (spermatogonia), daughter spermatoblasts (secondary spermatocytes), spermatids, and spermatozoa, but found them to be too small for any accurate observations. The spermatogonia lie nearest the entoderm, while the later stages occur further toward the outside of the gonad. The spermatozoa are found just under the layer of ectoderm. They are, presumably, shed by rupturing this thin superficial layer.

The spermatogonia form a closely packed layer nearest the entoderm. They are irregular cells, having a diameter of about six microns. The nuclei are large, filling a considerable part of the total volume of the cell. They have large deeply staining nucleoli, which are usually centrally placed. The nucleolus is generally single, although double ones occur. The nuclear membrane is delicate but can be readily seen under 1.5 oil immersion lens. The scarcity of mitotic figures among the spermatogonia indicates that division of these stages had stopped in the gonads under observation. The condition of the chromatin, packed into a single large nucleolus, seems to be the typical resting stage of the spermatogonia. Figure 1 shows a resting spermatogonium.

The first indication of the change from spermatogonia to primary spermatocytes is a fragmenting of the large nucleolus. It may break into several pieces which gradually diminish in size. In a number of cases, however, the nucleolus breaks into two portions which move apart within the nucleus before fragmentation begins. When this process does start, one of the pieces disappears before the other, leaving for a time a large dark staining body at one side of the nucleus and finely granular chromatin at the other. As fragmentation of the nucleolus continues the chromatin becomes finely granular and is practically unstainable with iron hæmatoxylin. It stains with safranin, however, in this condition. In cells adjacent to those in the granular stage, leptotene threads begin to appear. The chromatin at this stage forms knots which are suspended just under the nuclear membrane. The nucleus has attained its maximum size at this time. From this irregular network heavy threads develop. There is evidently an increase in the total quantity of chromatin, certainly in the stainable part, since the amount in the heavy syndesis mass shown in Fig. 6 is much greater than the delicate mesh shown in Fig. 5. The nucleus is not as large at this stage as it was in the preceding one.

The next change which can be observed is the disappearance of the nuclear membrane and a definite flattening of the mass to form

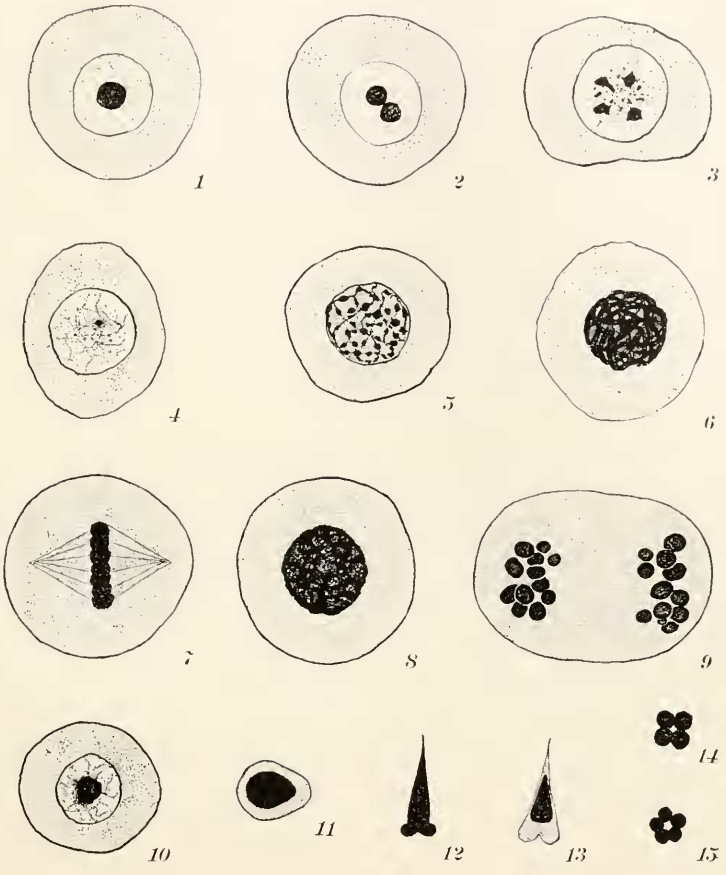
an equatorial plate. There are no central bodies visible, but spindle fibres are present. Because of the compactness of this equatorial plate, individual chromosomes are not readily distinguished at this stage. The plate soon splits and the chromosomes start a typical migration towards the poles of the cell. Only after this migration is nearly finished can individual chromosomes be observed. In this late anaphase twelve chromosomes have been counted in each end of a number of cells. The chromosomes are very small and are difficult to count, but there seem to be twelve at each pole at the late anaphase of the primary spermatocytes. Figure 9 is a diagrammatic sketch of the general arrangement of the chromosomes at this stage. They are not as clearly distinguishable as indicated in this figure.

The secondary spermatocytes are readily distinguished from the primary ones by their smaller size. At first the chromatin is in the form of a coarse densely packed mesh. It soon forms a compact knot which gradually changes to the equatorial plate of the spindle. The meiotic process continues without a pause, forming two daughter spermatids. The details of this division are difficult to observe because of the small size of the cells and the masking, caused by the compactness of the chromatin. The chromatin masses can be resolved into individual chromosomes only with great difficulty in the secondary spermatocytes. Figure 10 shows a secondary spermatocyte.

EXPLANATION OF PLATE

The figures of this plate, unless otherwise indicated, represent a magnification of 3500 diameters as they are reproduced. Figure 10 is drawn at a magnification of 4000 diameters. Figure 16 represents a magnification of 700 diameters. The figures were drawn with the aid of a 1.5 oil immersion (Spencer) and a 10 × ocular (Spencer). The drawings are all from sections.

FIG. 1. Resting spermatogonium showing large chromatin-nucleolus.
FIG. 2. Spermatogonium with double nucleolus.
FIG. 3. Primary spermatocyte showing fragmentation of the nucleolus.
FIG. 4. Primary spermatocyte with chromatin in finely granular state.
FIG. 5. Later stage of development.
FIG. 6. Pachytene stage.
FIG. 7. Metaphase of primary spermatocyte.
FIG. 8. Polar view of primary spermatocyte showing equatorial plate in which individual chromosomes are not readily distinguished.
FIG. 9. Diagrammatic sketch of late anaphase showing general arrangement of chromosomes. They are not as distinct as represented in this figure.
FIG. 10. Secondary spermatocyte with massed chromatin.
FIG. 11. Spermatid which has begun to elongate.
FIG. 12. Sperm stained with iron hæmatoxylin.
FIG. 13. Sperm stained by Flemming's triple method.
FIGS. 14 and 15. Axial views of the sperm in the region of the knobs.
FIG. 16. Section through the wall of the gonad. *a*. entoderm; *b*. mesogloea; *c*. spermatogonia; *d*. primary spermatocytes; *e*. secondary spermatocytes; *f*. spermatids; *g*. sperm; *h*. axial view of sperm; *i*. ectoderm.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The spermatids are at first small and round. They gradually draw out at one side. This continues until there is a distinct process projecting from one side, at the base of which are four or five balls or knobs. There are generally four knobs, but five are sometimes present. These knobs stain very intensely with iron hæmatoxylin, but stain only very slightly with safranin. The process takes iron hæmatoxylin stain, but not so intensely as the knobs, and is more readily destained. Figure 12 shows a sperm stained with iron hæmatoxylin. Figures 14 and 15 are axial views of the sperm in the region of the knobs, similarly stained. Figure 13 is a drawing of a sperm stained by Flemming's triple method. There is no indication of a flagellum in fixed material, but Vaney and Conte (1901) observed live sperm and reported the presence of a flagellum at the base of the knobs. If this observation is correct, the long pointed part is the anterior end of the sperm.

Summary

1. Fresh-water medusæ were observed in Stallworth Lake, an artificial body of water near Tuscaloosa, Alabama. They were first seen by the writer on September 14, 1928. They disappeared on October ninth of the same year and have not been observed since.

2. The hydroid stage was not found.

3. A description of the lake where the medusæ were found is given.

4. A historical survey of the fresh-water medusæ is made.

5. The medusæ agree very closely with *Craspedacusta ryderi* as described by Payne (1924). There are certain differences which may be developmental or due to dissimilar environmental conditions. The medusæ are assigned to the species *C. ryderi*.

6. The tentacles are grouped into four, instead of the three previously used groups.

7. Sections of the gonads demonstrated that all the medusæ examined were males.

8. Observations on the spermatogenesis of *C. ryderi* are included.

9. Since all gonads obtained were from adult medusæ, no information concerning the origin of the germ cells can be given.

10. The primary spermatocytes are only very slightly larger than the spermatogonia.

11. There is a meiotic stage in which the chromatin is stained only very poorly with iron hæmatoxylin.

12. There seem to be twelve chromosomes at each pole of the late anaphases of the primary spermatocytes.

13. Some portions of the sperm are stained more intensely by

Flemming's triple method and others by iron hæmatoxylin. Thus the two methods give different appearances to the sperm.

14. Tails are not visible on sperm in fixed and stained preparations.

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